PowerPlex® 1.2 System

Promega

Technical Manual No. D009

INSTRUCTIONS FOR USE OF PRODUCT DC6101. *PLEASE DISCARD PREVIOUS VERSIONS.*
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Printed in USA.
Revised 6/07

Part# TMD009

I. Description

STR[a] (short tandem repeat) loci consist of short, repetitive sequence elements 3 to 7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which can be detected using the polymerase chain reaction (5–8). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain or fluorescence detection following electrophoretic separation.

The PowerPlex® 1.2 System[a,b,c] allows the coamplification and two-color detection of nine loci (eight STR loci and Amelogenin). The system contains all the component STR loci of two *GenePrint*® quadriplex systems: the GammaSTR® System (D16S539, D7S820, D13S317 and D5S818) and the *GenePrint*® Fluorescent STR Multiplex—CSF1PO, TPOX, TH01 and vWA (CTTv multiplex), as well as the *GenePrint*® Fluorescent Sex Identification System—Amelogenin (TMR). One of the two primers for each locus in the GammaSTR® multiplex is labeled with fluorescein (FL); one primer is specific for each locus in the CTTv multiplex and one primer for Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR). All nine loci are amplified simultaneously in a single tube and analyzed in a single gel lane.

Note: Information on other Promega fluorescent STR systems and detection of amplified STR fragments using silver staining (9) is available on the Internet at: **www.promega.com** or upon request from Promega.

The PowerPlex® 1.2 System is designed specifically for use with the ABI PRISM® 310 Genetic Analyzer and is compatible with the ABI PRISM® 377 DNA Sequencer and ABI PRISM® 3100 Genetic Analyzer. The PowerPlex® 1.2 System provides all of the materials necessary for amplification of STR regions of purified genomic DNA with the exception of AmpliTaq Gold® DNA polymerase. This manual contains separate protocols for use of the PowerPlex® 1.2 System with the Perkin-Elmer model 480 and GeneAmp® PCR system 2400, 9600 and 9700 thermal cyclers in addition to protocols for separation of amplified products and detection of separated material. Protocols for operation of the fluorescence-detecting instrumentation should be obtained from the manufacturer.

II. STR Typing

II.A. Advantages of STR Typing

STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500bp long, much smaller than the material detected using AMP-FLP (10) or VNTR (11) analysis. STR typing is also amenable to a variety of rapid DNA purification techniques, which are compatible with PCR but do not provide enough DNA of appropriate quality for Southern blot-based analyses.

Promega STR amplification products are generally of discrete and separable lengths. This allows the construction of allelic ladders containing fragments of the same lengths as several or all known alleles for each locus. Visual or instrument-based comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles. Results obtained using the PowerPlex® 1.2 System can be recorded in a digitized format, allowing direct comparison with stored databases. Population analyses do not require the use of arbitrarily defined fixed bins for population data (12).

II.B. Advantages of Using the Loci in the PowerPlex® 1.2 System

The loci included in the PowerPlex® 1.2 System (Table 1) have a high degree of heterozygosity and display a minimum of artifacts. Each STR locus also displays alleles within a limited size range (Table 2). This allows simultaneous detection of several loci without overlap of alleles across loci. The sex identification locus, Amelogenin, is also included in the system. Table 3 lists the PowerPlex® 1.2 System allele determinations for commonly available standard DNA templates.

STR loci and primers were carefully selected to avoid or minimize artifacts, including those associated with *Taq* DNA polymerase such as repeat slippage and terminal nucleotide addition, as well as genetic artifacts called microvariant alleles. Repeat slippage (13,14), known as "n–4 bands", "stutter" or "shadow bands", is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA in sample material, or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being replicated. The loci chosen exhibit little or no repeat slippage. The vWA locus is an exception, revealing as much as 10% stutter. This locus has been included primarily for its popularity in the forensic DNA-testing community.

Terminal nucleotide addition (15,16) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the ends of amplified DNA fragments in a template-independent manner. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 30 minutes (17) to the amplification protocol to provide conditions of essentially full terminal nucleotide addition.

The presence of microvariant alleles complicates separation, interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (18,19). Therefore, we have selected loci with moderately high polymorphism and minimal occurrence of artifacts.

Table 1. The PowerPlex® 1.2 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Definition	Repeat Sequence[1] 5´→3´
Amelogenin	TMR	Xp22.10–22.3 and Y	HUMAMEL, Human Y chromosomal gene for Amelogenin-like protein	NA
D16S539	FL	16q24–qter	NA	AGAT
D7S820	FL	7q	NA	AGAT
D13S317	FL	13q22–q31	NA	AGAT
D5S818	FL	5q21–q31	NA	AGAT
CSF1PO	TMR	5q33.3–34	HUMCSF1PO, Human c-fms-proto-oncogene for CSF-1 receptor gene	AGAT
TPOX	TMR	2p23–2pter	HUMTPOX, Human thyroid peroxidase gene	AATG
TH01	TMR	11p15.5	HUMTH01, Human tyrosine hydroxylase gene	AATG
vWA	TMR	12p12–pter	HUMVWFA31, Human von Willebrand factor gene	AGAT

[1]Repeat sequences represent all four possible permutations (e.g., AGAT is used for AGAT, GATA, ATAG or TAGA). The first alphabetic representation of the repeat (e.g., AGAT) is employed according to Edwards *et al.* (2).

TMR = carboxy-tetramethylrhodamine

FL = fluorescein

NA = not applicable

Note: Amelogenin is not an STR but displays a 212-base, X-specific band and a 218-base, Y-specific band.

Table 2. The PowerPlex® 1.2 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[1]	Repeat Numbers of Alleles Not Present in Allelic Ladder
Amelogenin	TMR	212(X), 218(Y)	NA	None
D16S539	FL	264–304	5, 8–15	None
D7S820	FL	215–247	6–14	None
D13S317	FL	176–208	7–15	None
D5S818	FL	119–151	7–15	None
CSF1PO	TMR	291–327	6–15	None
TPOX	TMR	224–252	6–13	None
TH01	TMR	179–203	5–11	8.3[3], 9.3[3]
vWA	TMR	127–167	11, 13–21	None

[1]Lengths of each allele in the allelic ladders have been confirmed by sequence analyses.

[2]When using an internal lane standard such as the Fluorescent Ladder (CXR), 60–400 Bases, calculated sizes of allelic ladder components might differ from those listed.

[3]The TH01 allele 9.3 is quite common, while allele 8.3 is extremely rare. Other extremely rare variants have been reported at the TH01 locus.

Table 3. The PowerPlex® 1.2 System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates		
STR Locus	**K562**	**9947A**	**9948[1]**
D16S539	12,11	12,11	11,11
D7S820	11,9	11,10	11,11
D13S317	8,8	11,11	11,11
D5S818	12,11	11,11	13,11
CSF1PO	10,9	12,10	12,11,10
TPOX	9,8	8,8	9,8
Amelogenin	X,X	X,X	X,Y
TH01	9.3,9.3	9.3,8	9.3,6
vWA	16,16	18,17	17,17

[1]Strain 9948 displays three alleles at the CSF1PO locus.

Note: Information on strains 9947A, 9948 and K562 is available online at: locus.umdnj.edu/nigms

Strain K562 is available from the American Type Culture Collection, www.atcc.org (Manassas, VA)

II.C. Power of Discrimination

The eight STR loci amplified with the PowerPlex® 1.2 System provide powerful discrimination. Population statistics for these loci and their various multiplex combinations are displayed in Tables 4–6. These data were developed as part of a collaboration (20) with Genetic Design, Inc. (Greensboro, NC). Generation of these data included analysis of over two hundred individuals from African-American, Caucasian-American and Hispanic-American populations. For additional population data for STR loci, see references 21–25.

Table 4 shows the matching probability (26) for the PowerPlex® 1.2 System in various populations. The matching probability of the system ranges from 1 in 114,000,000 for Caucasian-Americans to 1 in 274,000,000 for African-Americans. The matching probability of the PowerPlex® 1.2 System in combination with the *GenePrint*® Fluorescent STR Multiplex—F13A01, F13B, FESFPS, LPL (FFFL multiplex) is 1 in 303,000,000,000 for Caucasian-Americans and 1 in 4,610,000,000,000 for African-Americans.

A measure of discrimination often used in paternity analyses is the paternity index (PI), a means for presenting the genetic odds in favor of paternity given the genotypes for the mother, child and alleged father (27). The typical PIs for the PowerPlex® 1.2 System and the PowerPlex® 1.2 System in combination with the FFFL multiplex are shown in Table 5. The PowerPlex® 1.2 System alone provides typical paternity indices exceeding 260 in each group, enough to satisfy routine requirements for paternity determination. When the FFFL multiplex is also included, the values exceed 2,600 in all groups.

An alternative calculation used in paternity analyses is the power of exclusion (27). This value, calculated for the PowerPlex® 1.2 System, exceeds 0.9969 in all populations tested (Table 6). In combination with the FFFL multiplex, the values exceed 0.99974, demonstrating the usefulness of these two systems for paternity analyses as well as for forensic determinations.

Table 4. Matching Probabilities of the PowerPlex® 1.2 System in Various Populations.

	Matching Probability		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
PowerPlex® 1.2 System (8 STR loci)	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8
PowerPlex® 1.2 System combined with FFFL multiplex (12 STR loci)	1 in 4.61×10^{12}	1 in 3.03×10^{11}	1 in 4.75×10^{11}

Table 5. Typical Paternity Indices of the PowerPlex® 1.2 System in Various Populations.

	Typical Paternity Index		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
PowerPlex® 1.2 System	498	260	319
PowerPlex® 1.2 System combined with FFFL multiplex	8,373	3,976	2,627

Table 6. Power of Exclusion of the PowerPlex® 1.2 System in Various Populations.

	Power of Exclusion		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
PowerPlex® 1.2 System (8 STR loci)	0.9982	0.9969	0.9973
PowerPlex® 1.2 System combined with FFFL multiplex (12 STR loci)	0.99990	0.99981	0.99974

II.D. The Fluorescent Ladder (CXR), 60–400 Bases

The Fluorescent Ladder (CXR), 60–400 Bases, is an internal lane standard that contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375 and 400 bases in length. Each fragment is labeled with carboxy-X-rhodamine (CXR) and can be detected separately (as a third color) in the presence of PowerPlex® 1.2 System-amplified material using the Applied Biosystems DNA sequencers. This ladder can be used as an internal size standard in each lane to increase precision in analyses when using the PowerPlex® 1.2 System. A protocol for preparation and use of this internal lane standard is provided in Sections VI.C, VII.B and VIII.E.

II.E. Warnings and Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality control measures that are not contained in this manual (28,29).

The quality of the purified DNA sample, as well as small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect the success of PCR. We suggest strict adherence to recommended procedures for amplification, as well as for denaturing gel electrophoresis and fluorescence detection.

STR analysis is subject to contamination by very small amounts of nontemplate human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, setting up amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (Gold ST★R 10X Buffer and PowerPlex® 1.2 10X Primer Pair Mix) should be stored separately from those used following amplification (PowerPlex® 1.2 Allelic Ladder Mix, Fluorescent Ladder (CXR), and Loading Solution). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips (e.g., ART® tips, Section XII.B).

Some of the reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Table 7 describes the potential hazards associated with such reagents.

Table 7. Hazardous Reagents.

Reagents for ABI PRISM® 310 and 3100 Genetic Analyzers	Hazard
formamide (contained in the Blue Dextran Loading Solution)	irritant, teratogen

Reagents for ABI PRISM® 377 DNA Sequencer	Hazard
acrylamide (Long Ranger® gel solution)	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
formamide (contained in the Blue Dextran Loading Solution)	irritant, teratogen
TEMED	corrosive, flammable
urea	irritant

III. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® 1.2 System	100 reactions	DC6101

Not For Medical Diagnostic Use. Cat.# DC6101 contains sufficient reagents for 100 reactions of 25µl each. Includes:

- 300µl Gold ST★R 10X Buffer
- 250µl PowerPlex® 1.2 10X Primer Pair Mix
- 25µl PowerPlex® 1.2 Allelic Ladder Mix
- 100µl *GenePrint*® TH01 Allele 9.3 (TMR)
- 125µl Fluorescent Ladder (CXR), 60–400 Bases
- 3µg K562 DNA High Molecular Weight (10ng/µl)
- 1ml Blue Dextran Loading Solution
- 1 Protocol

Storage Conditions: Store all components at –20°C. The PowerPlex® 1.2 10X Primer Pair Mix, PowerPlex® 1.2 Allelic Ladder Mix, TH01 Allele 9.3, and Fluorescent Ladder (CXR) must be stored in the dark. The postamplification components (Allelic Ladder Mix, TH01 Allele 9.3, Fluorescent Ladder (CXR), and Loading Solution) are packaged separately to prevent cross-contamination. We strongly recommend that pre-amplification and postamplification reagents be stored and used separately with different pipettes, tube racks, etc.

! The Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening.

Product	Cat.#
PowerTyper™ Macros	DG3470

Not For Medical Diagnostic Use. The PowerTyper™ Macros, for use with Genotyper® software, are available from Promega. This CD-ROM contains the file "PowerTyper™ 1.2 Macro" for use with the PowerPlex® 1.2 System. The Macros can also be downloaded at: **www.promega.com/geneticidtools/**

IV. DNA Extraction and Quantification Methods

The DNA IQ™ System (Cat.# DC6701) is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (30). This novel system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples such as blood or solutions. The DNA IQ™ Resin is designed to eliminate PCR inhibitors and contaminants frequently encountered in casework samples. For larger samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate and quantify DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples, and stains on support materials. The DNA IQ™ System has been tested with the PowerPlex® Systems to ensure a streamlined process. See Section XII.B for ordering information.

For applications requiring human-specific DNA quantification, the AluQuant® Human DNA Quantitation System (Cat.# DC1010) has been developed to work with the PowerPlex® Systems (31). See Section XII.B for ordering information.

Both the DNA IQ™ System and AluQuant® Human DNA Quantitation System have been fully automated on the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation (32). For information on automation of laboratory processes, on Beckman Coulter or other workstations, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com**) or e-mail: **techserv@promega.com**

V. Protocols for DNA Amplification Using the PowerPlex® 1.2 System

Materials to Be Supplied by the User

- thermal cycler model 480 or GeneAmp® system 2400, 9600 or 9700 (Applied Biosystems)
- microcentrifuge
- 0.5ml or 0.2ml (thin-walled) microcentrifuge tubes (Applied Biosystems)
- 1.5ml amber-colored microcentrifuge tubes (Fisher Cat.# 05-402-26)
- aerosol-resistant pipette tips (Section XII.B)
- AmpliTaq Gold® DNA polymerase (Applied Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- Mineral Oil (Cat.# DY1151, for use with the thermal cycler model 480)

We routinely amplify 1ng of template DNA in a 25µl reaction volume using the protocols detailed below. Amplification protocols for the Perkin-Elmer model 480 and the GeneAmp® PCR system 2400, 9600 and 9700 thermal cyclers are provided.

The PowerPlex® 1.2 System has been developed for amplification using AmpliTaq Gold® DNA polymerase and Gold ST★R 10X Buffer. AmpliTaq® DNA polymerase can be used with the Gold ST★R 10X Buffer and the PowerPlex® 1.2 System, but the balance of product yield among the loci might be less uniform than with the use of AmpliTaq Gold® DNA polymerase.

V.A. Amplification Setup

(!) Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section X.A.

The use of gloves and aerosol-resistant pipette tips is **highly** recommended to prevent cross-contamination. Keep all pre-amplification and postamplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

1. Thaw the Gold ST★R 10X Buffer and PowerPlex® 1.2 10X Primer Pair Mix.

   Notes:

   - It is very important to mix these reagents by vortexing for 5–10 seconds before each use. Do not centrifuge the 10X Primer Pair Mix, as this might cause the primers to be concentrated at the bottom of the tube.
   - A precipitate might form in the Gold ST★R Buffer. If this occurs, warm the buffer briefly at 37°C, then vortex until it is in solution.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does waste a small amount of each reagent, it ensures that you will have enough PCR master mix for all samples. It also ensures that each reaction contains the same master mix.

Note: If using the GeneAmp® PCR System 2400, 9600 or 9700 thermal cyclers, use 0.2ml thin-walled MicroAmp® reaction tubes. For the Perkin-Elmer Model 480, the standard 0.5ml GeneAmp® reaction tubes are recommended.

3. Place one clean, 0.2ml or 0.5ml microcentrifuge tube for each reaction into a rack, and label appropriately.

4. Calculate the required amount of each component of the PCR master mix (Table 8). Multiply the volume (µl) per sample by the total number of reactions (from Step 2) to obtain the final volume (µl).

5. In the order listed in Table 8, add the final volume of each reagent into a sterile, 1.5ml amber-colored tube. Mix gently. Place on crushed ice if using *Taq* DNA polymerase.

Table 8. Preparation of Master Mix for the PowerPlex® 1.2 System.

PCR Master Mix Component	Volume Per Sample	×	Number of Reactions	=	Final Volume (µl)
Nuclease-Free Water	17.05µl				
Gold ST★R 10X Buffer	2.5µl				
PowerPlex® 1.2 10X Primer Pair Mix	2.5µl				
AmpliTaq Gold® DNA polymerase[1]	0.45µl (2.25u)				
master mix volume	22.5µl				
Per Tube					
template DNA volume (to be added)	2.5µl				
total reaction volume	25µl				

[1]Assumes the AmpliTaq Gold® DNA polymerase is at 5u/µl. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.

Note: If the volume of AmpliTaq Gold® DNA polymerase added to the master mix is less than 0.5µl, you might wish to dilute the enzyme with 1X Gold ST★R Buffer first and add a larger volume. The amount of Nuclease-Free Water in the reaction should be adjusted accordingly so that the final volume of master mix per reaction is 22.5µl. **Do not** store diluted AmpliTaq Gold® DNA polymerase.

6. Add 22.5µl of PCR master mix to each reaction tube and place at room temperature (or on crushed ice if using *Taq* DNA polymerase).
7. Pipet 2.5µl (1ng template DNA) of each sample into the respective tube containing 22.5µl of PCR master mix.

   Note: If template DNA is stored in TE buffer, the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA), or other PCR inhibitors, which might be present at low concentrations depending on the source of template DNA and extraction procedure used.
8. For the positive amplification control, dilute the K562 DNA to 0.4ng/µl. Pipet 2.5µl (1ng) of diluted K562 DNA into a microcentrifuge tube containing 22.5µl of PCR master mix.
9. For the negative amplification control, pipet 2.5µl of Nuclease-Free Water (instead of template DNA) into a microcentrifuge reaction tube containing 22.5µl of the PCR master mix.
10. If using the GeneAmp® PCR system 2400, 9600 or 9700 thermal cycler and MicroAmp® reaction tubes, no addition of mineral oil to the reaction tubes is required. However, if using the model 480 thermal cycler and GeneAmp® reaction tubes, add 1 drop of mineral oil to each tube before closing.

Note: Allow the mineral oil to flow down the side of the tube and form an overlay to limit sample loss or cross-contamination due to splattering.

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V.B. Amplification Thermal Cycling

1. Place the tubes in a thermal cycler.
2. Select and run a recommended protocol. The preferred protocols for use with the GeneAmp® PCR system 9700, the GeneAmp® PCR system 9600, the GeneAmp® PCR system 2400 and the Perkin-Elmer model 480 thermal cyclers are provided below.
3. After completion of the thermal cycling protocol, store the samples at –20°C in a light-protected box.

! Storage of amplified samples at 4°C or higher can produce degradation products.

Note: Amplification and detection instrumentation can vary. You might need to optimize protocols including cycle number and injection time (or loading volume) for each laboratory instrument.

Note: PowerPlex® 1.2 is optimized for 1ng of DNA template.

In-house validation should be performed.

Protocol for the Perkin-Elmer GeneAmp® PCR System 9700 Thermal Cycler	**Protocol for the Perkin-Elmer GeneAmp® PCR System 2400 Thermal Cycler**
Ramp Speed: 9600 95°C for 11 minutes, then: 96°C for 1 minute, then: ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for **10 cycles**, then: ramp 100% to 90°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for **20 cycles**, then: 60°C for 30 minutes 4°C soak	95°C for 11 minutes, then: 96°C for 1 minute, then: ramp 100% to 94°C for 30 seconds ramp 100% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for **10 cycles**, then: ramp 100% to 90°C for 30 seconds ramp 100% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for **20 cycles**, then: 60°C for 30 minutes 4°C soak
Protocol for the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler	**Protocol for the Perkin-Elmer Thermal Cycler Model 480**
95°C for 11 minutes, then: 96°C for 1 minute, then: 94°C for 30 seconds ramp 68 seconds to 60°C (hold for 30 seconds) ramp 50 seconds to 70°C (hold for 45 seconds) for **10 cycles**, then: 90°C for 30 seconds ramp 60 seconds to 60°C (hold for 30 seconds) ramp 50 seconds to 70°C (hold for 45 seconds) for **20 cycles**, then: 60°C for 30 minutes 4°C soak	95°C for 11 minutes, then: 96°C for 2 minutes, then: 94°C for 1 minute 60°C for 1 minute 70°C for 1.5 minutes for **10 cycles**, then: 90°C for 1 minute 60°C for 1 minute 70°C for 1.5 minutes for **20 cycles**, then: 60°C for 30 minutes 4°C soak

! When using *Taq* DNA polymerase instead of AmpliTaq Gold® DNA polymerase, do not include the incubation at 95°C for 11 minutes prior to initiating the thermal cycling program.

V.C. Agarose Gel Electrophoresis of Amplification Products (Optional)

Agarose gel electrophoresis can be used to rapidly confirm the success of the amplification reaction prior to performing polyacrylamide gel or capillary electrophoresis.

Materials to Be Supplied by the User
(Solution compositions are provided in Section XII.A.)

- TAE 1X buffer
- agarose
- 5X loading solution
- ethidium bromide solution, 0.5µg/ml

1. Prepare a 2% agarose gel (approximately 150cm^2) by adding 2.0g of agarose to 100ml of TAE 1X buffer. Mark the liquid level on the container, then boil or heat in a microwave oven to dissolve the agarose. Add preheated (60°C) deionized water to make up for any volume lost due to evaporation.
2. Cool the agarose to 55°C before pouring into the gel tray. Be sure that the gel tray is level. Pour the agarose into the tray, insert the gel comb and allow to set for 20–30 minutes.
3. Prepare the samples by mixing 10µl of each amplified sample with 2.5µl of 5X loading solution.
4. Prepare 1 liter of TAE 1X buffer for the electrophoresis running buffer.
5. Place the gel and tray in the electrophoresis gel box. Pour enough running buffer into the tank to cover the gel to a depth of at least 0.65cm. Gently remove the comb.
6. Load each sample mixed with 5X loading solution (see Step 3, above).
7. Set the voltage at 5 volts/cm (measured as the distance between the two electrodes). Allow the gel to run for 2 hours.
8. After electrophoresis, stain the gel in TAE 1X buffer containing 0.5µg/ml ethidium bromide. Gently rock for 20 minutes at room temperature. Remove the ethidium bromide solution, and replace with deionized water. Allow the gel to destain for 20 minutes.
9. Photograph the gel using a UV transilluminator (302nm).

   Note: When analyzing the data, do not be alarmed if you see extra bands in addition to the alleles. DNA heteroduplexes can be expected when performing nondenaturing agarose gel electrophoresis. The sole purpose of the agarose gel is to confirm the success of the PCR.

VI. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer

Materials to Be Supplied by the User

(Solution compositions are provided in Section XII.A.)

- dry heating block, water bath or thermal cycler
- 310 capillaries, 47cm × 50µm (Applied Biosystems)
- performance optimized polymer 4 (POP-4™; Applied Biosystems)
- glass syringe (1ml)
- 10X genetic analyzer buffer with EDTA (Applied Biosystems)
- sample tubes and septa (Applied Biosystems)
- aerosol-resistant pipette tips (Section XII.B)
- Hi-Di™ formamide (Applied Biosystems Cat. #4311320)
- PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640)
- crushed ice

Note: To resolve the 9.3 and 10 alleles, the use of performance optimized polymer 6 (POP-6™) might be necessary.

Caution: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions when handling this substance. Always wear double gloves and safety glasses when working with formamide.

The quality of the formamide is critical. Use deionized formamide with a conductivity <100µS/cm. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause a breakdown of the formamide. Formamide with a conductivity >100µS/cm might contain ions that compete with DNA during injection. This results in lower peak heights and reduced sensitivity. A longer injection time might not increase the signal.

VI.A. Matrix Standardization

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 310 Genetic Analyzer. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer. For best results, the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121), should not be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

Regardless of which instrument or matrix standards are used, use only one JOE matrix fragment (i.e., JOE Matrix A or JOE Matrix B) when generating a matrix.

For protocols and additional information on matrix standardization, see the *PowerPlex® Matrix Standards, 310/377 Technical Bulletin* #TBD018, which is supplied with Cat.# DG3640. This manual is available upon request from Promega or at: **www.promega.com/tbs/**

VI.B. Instrument Preparation

1. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.
2. Open the ABI PRISM® 310 data collection software.
3. Prepare a GeneScan® sample sheet as described in the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Enter the appropriate sample information in the "sample info" column.

   For the red dye color, insert "ILS" (i.e., internal lane standard) to represent the Fluorescent Ladder (CXR), 60–400 Bases. For rows containing the PowerPlex® 1.2 Allelic Ladder Mix, insert "Ladder" in the "sample info" column for the blue dye color and yellow dye color. This information must be entered to successfully analyze your data using the PowerTyper™ 1.2 Macro (available online at: **www.promega.com/geneticidtools/** or upon request from Promega).

4. Create a new GeneScan® injection list. Select the appropriate sample sheet by using the pull-down menu.
5. Select the "GS STR POP4 (1ml) A" Module using the pull-down menu. Change the injection time to 2 seconds and keep the default settings for the remaining parameters as shown below:

Inj. Secs:	2
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time:	24

6. Select the appropriate matrix file (Section VII.A).
7. To analyze the data automatically, select the auto analyze checkbox and the appropriate analysis parameters and size standard. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for specific information on these options.

! You might need to optimize injection time at Step 5 for individual instruments. Injection times of 2–5 seconds are recommended for amplifications that contain 1ng of DNA template.

VI.C. Sample Preparation

The Fluorescent Ladder (CXR), 60–400 Bases, is included in the PowerPlex® 1.2 System as the internal lane standard (ILS) for three-color detection and analysis of amplified samples.

1. Prepare a loading cocktail by combining the Fluorescent Ladder (CXR) and Hi-Di™ formamide as follows:

   [(1µl Fluorescent Ladder) × (# injections)] + [(24µl Hi-Di™ formamide) × (# injections)]
2. Vortex for 10–15 seconds.
3. Combine 25µl of the prepared loading cocktail and 1µl of amplified sample. Mix by pipetting.

   Note: Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail might need to be increased or decreased. If the peak heights are too high (i.e., greater than 2,000RFU), the samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This might result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions.
4. Combine 25µl of the prepared loading cocktail and 1µl of the PowerPlex® 1.2 Allelic Ladder Mix (vortex the Allelic Ladder Mix prior to pipetting).

   Note: To run the TH01 Allele 9.3 alone, prepare a 1:4 dilution (1 part TH01 Allele 9.3 with 3 parts 1X STR Buffer), and combine 1µl of this dilution with 25µl of loading cocktail. To run the TH01 Allele 9.3 in combination with the PowerPlex® 1.2 Allelic Ladder, prepare a 1:3 mixture (1 part TH01 Allele 9.3 with 3 parts PowerPlex® 1.2 Allelic Ladder), then combine 1µl of this mixture with 25µl of loading cocktail.
5. Denature the samples and ladder by heating at 95°C for 3 minutes, and immediately chill on crushed ice for 3 minutes. Denature the samples just prior to loading.
6. Assemble the tubes in the appropriate autosampler tray (48- or 96-tube).
7. Place the autosampler tray in the instrument, and close the instrument doors.

Note: The PowerTyper™ 1.2 Macro is not compatible with ladder samples containing the TH01 Allele 9.3 and will give an error message so that no alleles are called.

VI.D. Capillary Electrophoresis and Detection

1. After loading the sample tray and closing the doors, select "Run" to start the capillary electrophoresis system.
2. Monitor the electrophoresis by observing the raw data and status windows.
3. Each sample will take approximately 30 minutes for syringe pumping, sample injection and sample electrophoresis.
4. Analyze the data using the GeneScan® Analysis Software. Amplified sample peak heights of less than 2,000RFU are ideal.

   Note: Peak heights greater than 2,000RFU might generate artifact peaks due to instrument saturation (i.e., overloading the sample). If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights might also appear less uniform.

VII. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer

Materials to Be Supplied by the User

- dry heating block, water bath or thermal cycler
- crushed ice
- aerosol-resistant pipette tips
- 3100 capillary array, 36cm
- performance optimized polymer 4 (POP-4™) for the 3100
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa for the 3100
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121)

Note: To resolve the 9.3 and 10 alleles, the use of performance optimized polymer 6 (POP-6™) might be necessary.

Caution: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions when handling this substance. Always wear double gloves and safety glasses when working with formamide.

The quality of the formamide is critical. Use Hi-Di™ formamide with a conductivity <100µS/cm. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause a breakdown of the formamide. Formamide with a conductivity >100µS/cm might contain ions that compete with DNA during injection. This results in lower peak heights and reduced sensitivity. A longer injection time might not increase the signal.

VII.A. Spectral Calibration

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 3100 Genetic Analyzer. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121), is required for spectral calibration on the ABI PRISM® 3100 Genetic Analyzer. The PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640), cannot be used for spectral calibration on this instrument.

VII.B. Sample Preparation

The Fluorescent Ladder (CXR), 60–400 Bases (Cat.# DG6221), is included with the PowerPlex® 1.2 System as the internal lane standard.

1. Prepare a loading cocktail by combining and mixing the internal lane standard and Hi-Di™ formamide as follows:

   [(1µl ILS) × (# injections)] + [(9µl Hi-Di™ formamide) × (# injections)]

2. Pipet 10µl of formamide/internal lane standard mix into each well.
3. Add 1µl of amplified sample (**or** 1µl of the Allelic Ladder Mix). Cover wells with appropriate septa.

   Note: Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail might need to be increased or decreased. If the peak heights are too high (>2,000RFU), the samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This can result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles.

4. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice for 3 minutes. Denature the samples just prior to loading the ABI PRISM® 3100 Genetic Analyzer.

Note: The volume of internal lane standard used in the loading cocktail can also be increased or decreased to adjust the intensity of the size standard peak heights.

Note: Brief centrifugation of prepared samples will remove bubbles that might affect analysis.

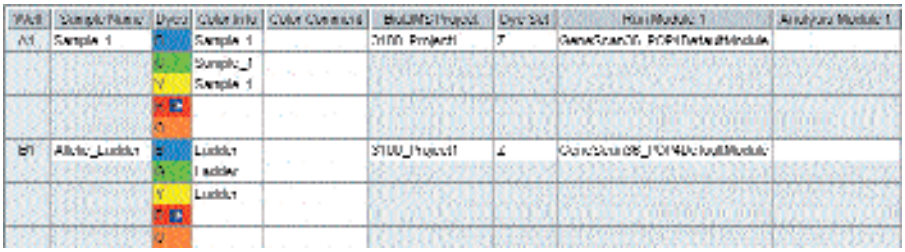
VII.C. Instrument Preparation

1. Refer to the *ABI PRISM® 3100 Genetic Analyzer User's Manual* for instructions on cleaning the pump blocks, installing the capillary array, performing a spatial calibration, and adding polymer to the reserve syringe.
2. Open the ABI PRISM® 3100 data collection software.
3. Open a new plate record. Name the plate, and select "GeneScan". Select the plate size (96-well or 384-well). Click "Finish".
4. Complete the plate record spreadsheet for the wells you have loaded. Enter appropriate information into the "Sample Name" and "Color Info" columns. For Allelic Ladder samples, insert the word "ladder" into the "Color Info" column for the blue, yellow, and green dye colors. This information must be entered to successfully analyze data with the PowerTyper™ 1.2 Macro.
5. In the "BioLIMS Project" column, select "3100_Project1" from the pull-down menu.
6. In the "Dye Set" column, select "Z" from the pull-down menu.
7. In the "Run Module 1" column, select "GeneScan36_POP4DefaultModule" from the pull-down menu.
8. To collect the data without autoanalyzing, select "No Selection" in the "Analysis Module 1" column. Analysis parameters can be applied after data collection and during data analysis using the GeneScan® analysis software.

   To analyze the data during data collection, an appropriate analysis module must be selected in the "Analysis Module 1" column. Refer to the *ABI PRISM® 3100 Genetic Analyzer User's Manual* for specific instructions on creating analysis modules.

Note: If less signal intensity is desired, injection time can be decreased to 5 seconds in the run module. Use the "Module Editor" under "Tools" to change the run module.

The resulting GeneScan® plate record should appear as follows:



9. Click "OK". This new plate record will appear in the pending plate records table on the plate setup page of the collection software.
10. Place samples in instrument, and close the instrument doors.
11. In the pending plate records table, click once on the name of the plate record you just created.
12. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.
13. When the plate record is linked to the plate, the plate graphic will change from yellow to green, the plate record moves from the pending plate records table to the linked plate records table, and the "Run Instrument" button becomes enabled.
14. Click the "Run Instrument" button on the toolbar to start the sample run.
15. Monitor electrophoresis by observing the run, status, array and capillary views windows in the collection software. Each run (16 samples/capillaries) will take approximately 45 minutes.

VII.D. Sample Detection

1. Analyze the data using the GeneScan® analysis software.
2. Review the raw data for one or more sample runs. Highlight the sample file name, then under the "sample" menu select "raw data." Move the cursor so the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.
3. The recommended analysis parameters are:

Analysis Range	Start: Defined in Step 2 Stop: 10,000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light[1]
Peak Detection	Peak Amplitude Thresholds[2]: B: Y: G: R: Min. Peak Half Width: 2pts
Size Call Range	Min: 60 Max: 600
Size Calling Method	Local Southern Method
Split Peak Correction	None

[1]Smoothing options should be determined by individual laboratories. Occasionally the separation control alleles and the TH01 alleles 9.3 and 10 will not be distinguished using heavy smoothing.

[2]The peak amplitude thresholds are the minimum peak height that the software will call as a peak. Values for the peak amplitude thresholds are usually 50–200RFU and should be determined by individual laboratories.

4. The analysis parameters can be saved in the "Params" folder.
5. Apply the stored analysis parameters file to the samples.
6. Assign a new size standard. Select a sample file, highlight the arrow next to the size standard, then select "define new." Assign the size standard peaks as shown in Figure 1. Store the size standard in the "SizeStandards" folder.

   Note: If pull-up or bleedthrough from TMR (yellow) is seen in the CXR (red) channel, the peak amplitude threshold can be increased (i.e., 100–200RFU) for the red channel in the analysis parameters. This does not interfere with interpretation, as the CXR (ILS) fragments should be greater than 300RFU.
7. Apply the size standard file to the samples, then analyze the sample files.
8. See Section IX for further data analysis.

For additional information regarding the GeneScan® analysis software, refer to the *GeneScan® Analysis Software User's Manual*.

Notes:

- Amplified sample peak heights <2,000RFU are ideal.
- Peak heights >2,000RFU might generate artifact peaks due to instrument saturation (i.e., overloading the sample). Bleedthrough (pull-ups) or oversubtraction (pull-downs) from one color to another might be observed. Saturated signal might also appear as two small peaks.
- If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights might also appear less uniform.
- There might be variation between instruments regarding the relative fluorescence units detected using the same sample. Furthermore, different instruments vary in the relative efficiency of color detection, affecting the dye color-to-dye color balance.

VIII. Detection of Amplified Fragments Using the ABI PRISM® 377 DNA Sequencer

Materials to Be Supplied by the User

(Solution compositions are provided in Section XII.A.)

- dry heating block, water bath or thermal cycler
- Long Ranger® gel solution (BioWhittaker Molecular Applications Cat.# 50611) or Long Ranger Singel® pack for ABI sequencers 377-36cm (BioWhittaker Molecular Applications Cat.# 50691)
- 10% ammonium persulfate (Cat.# V3131)
- TEMED
- Urea (Cat.# V3171)
- TBE 10X buffer
- Nalgene® tissue culture filter (0.2 micron)
- aerosol-resistant pipette tips (Section XII.B)
- gel-loading pipette tips
- 36cm front and rear glass plates
- 36cm gel spacers (0.2mm thick)
- 36-well sharkstooth comb or 34-well squaretooth comb (0.2mm thick)
- clamps (e.g., large office binder clamps)
- Liqui-Nox® detergent
- crushed ice

! Acrylamide is a neurotoxin. Always wear double gloves and safety glasses when handling.

VIII.A. Polyacrylamide Gel Preparation

Acrylamide (Long Ranger® gel solution) is a neurotoxin and suspected cancer agent; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions. Always wear gloves and safety glasses when working with acrylamide solutions.

The following protocol is for preparation of a 36cm denaturing polyacrylamide gel for use with the ABI PRISM® 377 DNA sequencer. Low-fluorescence glass plates are recommended and can be obtained from the instrument manufacturer.

1. Thoroughly clean the glass plates with hot water and a 1% Liqui-Nox® solution. Rinse extremely well using deionized water. Allow the glass plates to air-dry in a dust-free environment.
2. Assemble the glass plates by placing 0.2mm side gel spacers between the front and rear glass plates. Hold the plates together using binder clamps (4 clamps on each side). Place the assembly horizontally on a test tube rack or similar support.
3. Prepare a 5% Long Ranger® acrylamide gel (total of 50ml) by combining the ingredients listed in Table 9. Stir the solution until the urea has dissolved.

Table 9. Preparation of a 5% Long Ranger® Polyacrylamide Gel.

Component	5% Gel	Final Concentration
urea	18g	6M
deionized water	26ml	—
10X TBE buffer	5ml	1X
50% Long Ranger® gel solution	5ml	5%
total volume	50ml	

Note: Long Ranger Singel® Packs can be used.

4. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter), and degas for an additional 5 minutes.
5. Add 35µl of TEMED and 250µl of fresh 10% ammonium persulfate to the 50ml of acrylamide solution, and mix gently.
6. Using a disposable 30cc syringe, pour the gel by starting at the well end of the plates and carefully injecting the acrylamide between the horizontal glass plates. Allow the solution to fill the top width of the plates. While maintaining a constant flow of solution, gently tap the glass plates to assist the movement of solution to the bottom of the plates and to prevent the formation of bubbles.
7. Insert a 36-well sharkstooth comb or 34-well squaretooth comb between the glass plates. Sharkstooth combs with 64 or 96 wells can also be used.
8. Secure the comb with 3 evenly spaced clamps.
9. Keep the remaining acrylamide solution as a polymerization control.
10. Allow polymerization to proceed for at least 2 hours. Check the polymerization control to be sure that polymerization has occurred.

Note: The gel can be stored overnight if a paper towel saturated with deionized water and plastic wrap are placed around the top and bottom to prevent the gel from drying out (crystallization of the urea will destroy the gel).

VIII.B. Matrix Standardization

Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 377 DNA Sequencer. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 310/377 (Cat.# DG3640), is required for matrix standardization for the ABI PRISM® 377 DNA Sequencer. For best results, the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121), should not be used to generate a matrix on the ABI PRISM® 377 DNA Sequencer.

For protocols and additional information on matrix standardization, see the *PowerPlex® Matrix Standards, 310/377 Technical Bulletin* #TBD018, which is supplied with Cat.# DG3640. This manual is available upon request from Promega or at: **www.promega.com/tbs/**

VIII.C. Instrument Preparation

1. Open the ABI PRISM® 377 data collection software.
2. Prepare a sample sheet as described in the *GeneScan® Analysis Software User's Manual*. Enter the appropriate sample information in the "sample info" column.

   For the red dye color, insert "ILS" (i.e., internal lane standard) to represent the Fluorescent Ladder (CXR), 600–400 Bases. For rows containing the PowerPlex® 1.2 Allelic Ladder Mix, insert "Ladder" for the blue dye color and yellow dye color. This information must be entered to successfully analyze your data using the PowerTyper™ 1.2 Macro.
3. Create a new GeneScan® run, and use the following settings:

Plate Check Module:	Plate Check A
PreRun Module:	PR GS 36A-2400
Run Module:	GS 36A-2400
Collect Time:	2.5 hours
Well-to-Read Distance:	36cm

4. Select the appropriate sample sheet and comb selection by using the pull-down menus.
5. Select the appropriate gel matrix file (Section VIII.B).

VIII.D. Gel Prerun

1. Remove the clamps from the polymerized acrylamide gel. If necessary, clean any excess acrylamide from the glass plates with paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb, and remove the comb. If using a sharkstooth comb, carefully insert the sharkstooth comb teeth into the gel approximately 1–2mm.
3. Position the gel/glass plate unit in the 377 cassette.
4. Secure the cassette in the instrument, and perform a plate check as recommended in the *ABI PRISM® 377 DNA Sequencer User's Manual*. If the horizontal line graph is not flat, remove the cassette, clean the plate surface, and repeat the plate check.
5. Add TBE 1X buffer to the top and bottom buffer chambers of the instrument.

6. Using a 30cc syringe filled with buffer, remove any air bubbles from the well area of the gel and place the lid on the upper buffer chamber. Using a syringe with a bent 19-gauge needle, remove any air bubbles from the bottom of the gel.
7. Attach the heating plate, connect the water tubing, attach all electrodes, close the instrument door, and select "PreRun". Allow the gel to prerun for 15–20 minutes or until the gel temperature is at least 40°C. Open the status window to monitor the temperature of the gel.
8. Prepare the sample and allelic ladder samples during the gel prerun.

VIII.E. Sample Preparation and Loading

The Fluorescent Ladder (CXR), 60–400 Bases, is included in the PowerPlex® 1.2 System as the internal lane standard (ILS) for three-color detection and analysis of amplified samples. With this approach, only 2–3 lanes of the PowerPlex® 1.2 Allelic Ladder Mix are required per gel.

1. Prepare a loading cocktail by combining and vortexing the Fluorescent Ladder (CXR) and Blue Dextran Loading Solution as follows:

   [(0.5µl Fluorescent Ladder) × (# lanes)] + [(1.5µl Blue Dextran Loading Solution) × (# lanes)]
2. Vortex for 10–15 seconds.
3. Combine 2µl of the prepared loading cocktail and 0.5–1µl of amplified sample. Mix by pipetting.

   Note: Instrument detection limits vary; therefore, the amount of product mixed with loading cocktail might need to be increased or decreased. If the peak heights are too high (i.e., greater than 2,000RFU), the samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This might result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles (i.e., 10/18 or 10/16 cycling).
4. Combine 2µl of the prepared loading cocktail and 1µl of the PowerPlex® 1.2 Allelic Ladder Mix (vortex the Allelic Ladder Mix prior to pipetting).

   Note: To run the TH01 Allele 9.3 alone, prepare a 1:4 dilution (1 part TH01 Allele 9.3 with 3 parts 1X STR Buffer), and combine 1µl of this dilution with 2µl of loading cocktail. To run the TH01 Allele 9.3 in combination with the PowerPlex® 1.2 Allelic Ladder, prepare a 1:3 mixture (1 part TH01 Allele 9.3 with 3 parts PowerPlex® 1.2 Allelic Ladder), then combine 1µl of this mixture with 2µl of loading cocktail.
5. Briefly spin the samples in a microcentrifuge to bring the contents to the bottom of the tubes.
6. Denature the samples by heating at 95°C for 2 minutes, and immediately chill on crushed ice. Denature the samples just prior to loading the gel.
7. After the 15- to 20-minute prerun, pause the instrument by clicking on the "pause" button. By pausing the prerun, the water will continue to circulate to keep the gel warm during the sample loading.
8. Use a 30cc syringe filled with buffer to flush the urea from the well area.
9. Load 1µl of each denatured sample into the respective wells.
10. Place the lid on the upper buffer chamber, and close the instrument door.

! You might need to optimize Step 9 for individual instruments. Loading volumes of 1.0–2.0µl are recommended.

VIII.F. Gel Electrophoresis and Detection

1. After loading, select "Cancel" to stop the prerun. Make sure that the run time is set at 2.5 hours, then select "Run" to begin electrophoresis.
2. Monitor the electrophoresis by observing the gel image and status windows.
3. Allow electrophoresis to proceed for 2.5 hours. The 400-base fragment will have migrated past the laser.
4. Analyze the gel according to the *GeneScan® Analysis Software User's Manual.* Amplified sample peak heights of less than 2,000RFU are ideal.

Notes:

- Peak heights greater than 2,000RFU can generate artifact peaks due to instrument saturation (i.e., overloading the sample). If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights might also appear less uniform.
- There might be variation between instruments regarding the relative fluorescence units detected using the same sample. Furthermore, the different instruments vary in the relative efficiency of color detection, affecting the dye color-to-dye color balance.

VIII.G. Reuse of Glass Plates

Separate the glass plates, and discard the gel. Clean the glass plates with hot water and a detergent such as 1% Liqui-Nox® detergent. Rinse extremely well with deionized water, and allow the plates to air-dry. Do not scrape the plates with abrasive materials during this process.

Note: Soap and oil can build up on plates, resulting in gel extrusion or hazy background. Plates can be soaked in 2N HCl for 15 minutes, then rinsed thoroughly.

IX. Data Analysis

IX.A. PowerTyper™ 1.2 Macro

To facilitate analysis of the data generated with the PowerPlex® 1.2 System, we have created a file to allow automatic assignment of genotypes using the Genotyper® software. After samples have been amplified using the PowerPlex® 1.2 System, detected using the ABI PRISM® 310 or 3100 Genetic Analyzer or 377 DNA sequencer, and analyzed using the GeneScan® analysis software, the sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ 1.2 Macro.

The PowerTyper™ 1.2 Macro is provided on the PowerTyper™ Macros CD-ROM (Cat.# DG3470), available upon request from Promega. PowerTyper™ files can also be downloaded from our web site at: **www.promega.com/geneticidtools/**

The PowerTyper™ 1.2 Macro is used in conjunction with Genotyper® 2.0 or 2.5 (Macintosh®) and Genotyper® 3.6 (Windows®) software. Therefore, the appropriate version of Genotyper® software must be installed on your computer.

Amplified sample peak heights of less than 2,000RFU are ideal. Be certain the "sample info" column for each lane containing the PowerPlex® 1.2 Allelic Ladder Mix contains the word "ladder". The macro uses the word "ladder" to identify the sample files containing allelic ladder. "Sample info" can be added or modified after importing into PowerTyper™ Macro. Highlight the sample, then select "show dye/lanes window" under "views."

The PowerTyper™ Macro is a Genotyper® file and can be overwritten if "save" is used instead of "save as".

The PowerPlex® 1.2 System contains a vial of *GenePrint*® TH01 Allele 9.3 (TMR) to enable the user to evaluate the resolution of TH01 alleles 9.3 and 10 using their conditions and detection instrumentation. The PowerTyper™ 1.2 Macro uses the PowerPlex® 1.2 Allelic Ladder Mix without the TH01 Allele 9.3 for determining sample genotypes. While the allelic ladder mix does not contain TH01 Allele 9.3, the macro will identify samples containing those alleles. We recommend mixing the TH01 Allele 9.3 with the PowerPlex® 1.2 Allelic Ladder Mix (Section VI.C or VIII.E) and running this mixture in one lane to demonstrate that the separation of TH01 alleles 9.3 and 10 is detected using your parameters.

Follow the instructions below to determine allele designations for the nine loci (D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, Amelogenin, TH01 and vWA) contained in the PowerPlex® 1.2 System. Each macro must be completed in the proper order to correctly genotype the allelic ladders and samples.

Note: Data generated on the ABI PRISM® 3100 Genetic Analyzer is analyzed using a Windows NT® version of the GeneScan® analysis software. This data can be imported directly into the Windows NT® Genotyper® version. Before this data can be imported into the Macintosh® version of Genotyper®, a conversion utility (available from Applied Biosystems) must be run. This utility enables the Windows NT®-generated data to be recognized by the Macintosh® software.

IX.B. Analysis

Important: The transferred file can be overwritten if "save as" is not used. This can be avoided by locking the file.

1. Transfer the PowerTyper™ 1.2 Macro from the PowerTyper™ Macros CD-ROM (Cat.# DG3470) to a designated location on your computer hard drive.
2. Open the Genotyper® software and the PowerTyper™ 1.2 Macro. For questions regarding the Genotyper® software, refer to the *Genotyper® User's Manual.*
3. Under "File", select "Import" to import the GeneScan® project or sample files to be analyzed. Import the blue, yellow and red dye colors. To import the red channel data, select "Import" from "Preferences" under the "Edit" menu.
4. Double-click on **Check ILS** macro. (The macros are listed at the bottom left corner of the active window.) A plots window will be displayed to show the internal lane standard (i.e., Fluorescent Ladder (CXR), 60–400 Bases) in the red dye color. Scroll down to view and edit all injections/lanes. If necessary, use the GeneScan® software to redefine the internal lane standard fragments and reanalyze the samples.

   Note: If the yellow dye color contains peak heights greater than 2,000RFU for the TMR-labeled loci (i.e., CTATv loci), bleedthrough (generally less than 150RFU) will be observed in the red dye color. This does not affect interpretation and allele designations of loci in the blue and yellow dye colors.
5. Double-click on the **POWER** macro. The offsets will be calculated for all loci. When completed, a plots window will open to display the blue dye allelic ladders (i.e., GammaSTR® System allelic ladders, D16S539, D7S820, D13S317 and D5S818). Confirm the correct allele numbers were assigned to the allelic ladders.

   In general, the allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Table 1. Analysis using GeneScan® analysis software and Genotyper® software allows allele determination by comparing amplified sample fragments with allelic ladders and internal lane standards.

When using an internal size standard, the calculated lengths of the allelic ladder components will differ from those listed in the table. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard and is not a matter of concern.

6. Double-click on the **CTATv Ladders** macro. A plots window will open to display the yellow dye allelic ladders (i.e., CTATv allelic ladders, CSF1PO, TPOX, Amelogenin, TH01 and vWA). Confirm that the correct allele numbers were assigned to the allelic ladders.
7. Double-click on the **Display GammaSTR®** macro to display the blue dye for all sample injections/lanes. Scroll down to observe and/or edit as necessary.
8. Double-click on the **Display CTATv** macro to display the yellow dye for all sample injections/lanes. Scroll down to observe and/or edit as necessary.

Notes:

- If samples are overloaded, a possible off-ladder allele might be detected in the vWA allele size range approximately 19–20 bases smaller than the allele, representing a homozygous sample.
- When using the ABI PRISM® 377 DNA Sequencer and samples are overloaded, broad and small (less than 100RFU) TMR-labeled peaks might be observed at approximately 122 and 156 bases.

9. Create the desired table by selecting the PowerTable 310 (for ABI PRISM® 310 or 3100 Genetic Analyzer data) or PowerTable 377 (for ABI PRISM® 377 DNA Sequencer data). Two additional table formats are available as macros, Make Allele Table or Make CODIS Table. You can customize these tables to fit the needs of your laboratory.

IX.C. Controls

1. Observe the lanes containing the negative controls. They should be devoid of amplification products.
2. Observe the lanes containing the K562 DNA positive controls. Amplified sample peak heights of less than 2,000RFU are ideal. Compare the K562 DNA allelic repeat sizes with the locus-specific allelic ladder. The expected K562 DNA allele sizes for each locus are listed in Table 3 (Section II).

IX.D. Results

Figure 1 shows typical results achieved using the PowerPlex® 1.2 System and the ABI PRISM® 310 Genetic Analyzer.

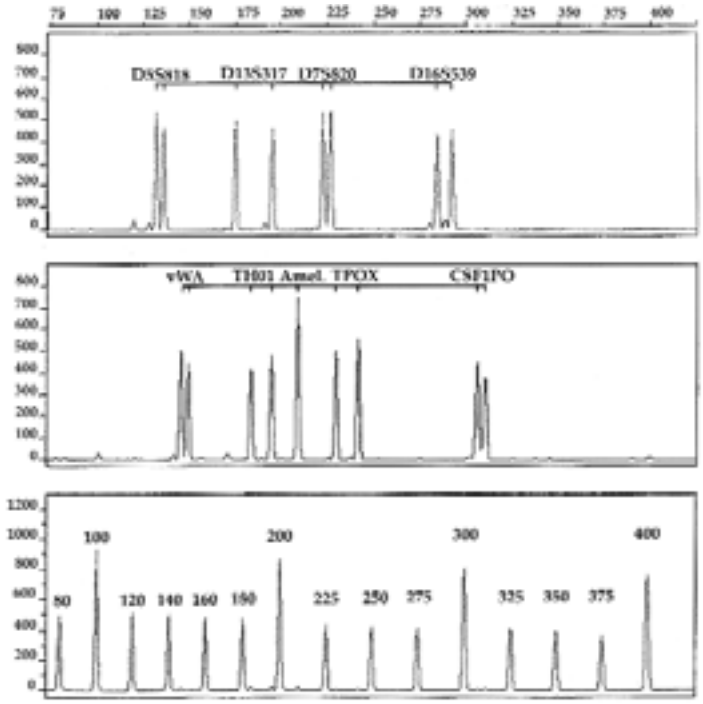


Figure 1. Electrophoretic profile of a single DNA sample amplified using the PowerPlex® 1.2 System and detected with the ABI PRISM® 310 Genetic Analyzer. The fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818) are displayed in the top panel. The TMR-labeled loci (CSF1PO, TPOX, Amelogenin, TH01 and vWA) are displayed in the middle panel. The Fluorescent Ladder (CXR), 600–400 Bases, is shown in the bottom panel and is used as the internal lane standard.

X. Troubleshooting

X.A. Amplification and Fragment Detection

Symptoms	Possible Causes	Comments
Faint or no allele peaks	Impure template DNA	Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used, inhibitors might be present in the DNA sample.
	Insufficient template DNA	Use the recommended amount of template DNA.
	Insufficient enzyme activity	Use the recommended amount of AmpliTaq Gold® DNA polymerase. Check the expiration date on the tube label.
	Incorrect amplification program	Confirm the amplification program.
	High salt concentration or altered pH	The DNA volume should not be more than 20% of the total reaction volume. Carryover from the DNA sample of K^+, Na^+, Mg^{2+} or EDTA can have a deleterious effect on PCR. A change in pH might also affect PCR.
	Thermal cycler or tube problems	Review the thermal cycling protocols in Section V.B. We have not tested other reaction tubes or thermal cyclers. Calibration of the thermal cycler heating block might be required.
	Primer concentration too low	Use the recommended primer concentration. Mix well before use.
	Insufficient mixing of reagents	Vortex all reagents and cocktails before use.
	Samples not denatured before loading	Make certain the samples are heat-denatured and cooled on ice immediately prior to loading the gel or capillary.
	Poor capillary electrophoresis injection [Fluorescent Ladder (CXR) also affected]	Re-inject the sample. Check the syringe for leakage. Check the laser power.
	Poor-quality formamide used	Ensure that high-quality formamide is used when running samples on the ABI PRISM® 310 and 3100 Genetic Analyzers. The conductivity of the deionized formamide should be <100µS/cm.

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X.A. Amplification and Fragment Detection (continued)

Symptoms	Possible Causes	Comments
Extra peaks visible in one or all of the color channels	Contamination with another template DNA or previously amplified DNA	Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Artifacts of STR amplification	PCR amplification of STR systems sometimes generates artifacts that appear as faint peaks one or four bases below an allele. Stutter band peak heights will be high if the samples are overloaded (i.e., >2000RFU).
	Samples not completely denatured	Heat-denature the samples for the recommended time, and cool on ice immediately prior to loading the gel or capillary.
	Background	Load less amplification product or decrease the injection time.
	Capillary electro-phoresis- (CE) related artifacts (“spikes”)	Minor voltage changes or urea crystals passing by the laser can cause “spikes” or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject the samples to confirm.
	CE-related artifacts (contaminants)	Contaminants in the water used on the ABI PRISM® 310 and 3100 Genetic Analyzers and to dilute the 10X genetic analyzer buffer can generate peaks in the blue and green dye colors. Use autoclaved water, change vials and wash buffer reservoir.
	Excessive amount of DNA	We recommend 1–2ng of DNA template. Amplification of DNA templates >2ng can result in a higher number of stutter bands. Use less DNA template, or reduce the number of cycles in the ampli-fication program by 2–4 cycles (10/18 or 10/16 cycling).
	Pull-up or bleedthrough	Pull-up can occur when peak heights are >2,000RFU or if a poor or incorrect matrix has been applied to the samples. Increase the peak amplitude threshold (i.e., 150–200RFU) in the analysis parameters, and reanalyze using the GeneScan® software. Generate a new matrix, and apply to the samples.

Symptoms	Possible Causes	Comments
Allelic ladder is not running the same as the sample	Allelic ladder and primer pair mix not compatible	Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Poor-quality formamide used	Ensure that high-quality formamide is used when running samples on the ABI PRISM® 310 and 3100 Genetic Analyzers. The conductivity of the deionized formamide should be <100µS/cm.
	Buffer incompatibility	Samples were diluted in the wrong buffer. Use 1X Gold ST★R Buffer to dilute samples.
	Migration of samples can change slightly over the course of a capillary electrophoresis (CE) run with many samples. This might be due to changes in temperature or in the CE column over time.	Use a different injection of the allelic ladder to determine sizes in the PowerTyper™ 1.2 Macro.
Imbalance of peak heights	Excessive amount of DNA	We recommend 1–2ng of DNA template. Amplification of DNA templates at greater than 2ng results in an imbalance in yields with the smaller loci showing more amplification product than the larger loci. Use less DNA template or reduce the number of cycles in the amplification program by 2–4 cycles (10/18 or 10/16 cycling) to improve locus-to-locus balance.
	Use of FTA® paper	Results might be similar to use of excess amounts of DNA template. Reduce the number of cycles in the amplification program by 2–4 cycles (10/18 or 10/16 cycling) to improve locus-to-locus balance.
	Degraded DNA sample	DNA template is degraded, and the larger loci show diminished yield.
	Insufficient template DNA	Use the recommended amount of template DNA.
	Poor-quality formamide used	Ensure that high-quality formamide is used when running samples on the ABI PRISM® 310 and 3100 Genetic Analyzers. The conductivity of the deionized formamide should be <100µS/cm.

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Note: Dilution of over-amplified samples displays dropoff of larger loci.

X. Troubleshooting (continued)

X.B. PowerTyper™ 1.2 Macro

Symptoms	Possible Causes	Comments
File does not open	Genotyper® software not installed	Be certain that Genotyper® 2.0 or later version is installed on your computer.
	Incorrect version of Genotyper® software	The PowerTyper™ 1.2 Macro might not work with versions prior to Genotyper® version 2.0.
	Damaged CD-ROM	The CD-ROM might have been damaged during shipment. Contact Technical Services at 800-356-9526 or 608-274-4330.
Error message: "Could not complete the "Run Macro" command because no dye/lanes are selected"	Allelic ladder sample files have not been identified	Be certain the "sample info" or "color info" column for each lane containing the PowerPlex® 1.2 Allelic Ladder Mix contains the word "ladder" to identify the sample files containing allelic ladder.
	All four dye colors not imported	For Genotyper® 2.5, set the preferences (under "edit") to import the blue, green, yellow and red colors.
The plots window or allele table does not display all the data	The macros were not run in the proper order	The Check ILS macro and Power macro must be run first before the remaining macros are run.
	All four dye colors not imported	For Genotyper® 2.5 or higher, set the preferences (under "edit") to import the blue, green, yellow and red colors.
The "Check ILS" macro displays an empty plot window	All four dye colors not imported	For Genotyper® 2.5 or higher, set the preferences (under "edit" to import the blue, green, yellow and red colors.
Many off-ladder peaks in the samples using the ABI PRISM® 310 or 3100 Genetic Analyzer	Migration of samples might change slightly over the course of a CE run with many samples. This might be due to changes in temperature or in the CE column over time	Use a different injection of the allelic ladder to determine sizes in the PowerTyper™ 1.2 Macro. Do not use the first injection on a new column for the ladder sample.
	The base pair size of the alleles is incorrect due to incorrect fragment sizes assigned to the internal lane standard	Confirm that the internal lane standard fragments are assigned correctly. Reanalyze the sample using GeneScan® software, and redefine the internal lane standard fragments.

Symptoms	Possible Causes	Comments
Error message: "Could not complete the "Run Macro" command because the labeled peak could not be found	The peak heights for one or more of the allelic ladder sample files are below 200RFU	The allelic ladder categories are defined as having a minimum peak height of 200RFU. If the ladder alleles are below 200RFU, the software will not be able to locate the allele peak.
	The TH01 9.3 allele was included in the "ladder" sample	Run the allelic ladder sample without the TH01 9.3 allele.
Error message: "Could not complete the "Run Macro" command because no dye/lanes are selected	The base pair size of the alleles within the allelic ladder does not not fall within the defined category range	Compare the size of the smallest allele in the allelic ladder with the base pair size and range listed in the same alleles. If necessary, increase the range to greater than ±4bp and save the new parameters.
		Confirm that the internal lane standard fragments are correctly sized. Redefine the internal lane standard fragments, and reanalyze the sample using GeneScan® software.
	Allelic ladder data is not compatible with the PowerTyper™ file used	Confirm that the PowerTyper™ Macro file matches the allelic ladder being used.
	CE spikes in the allelic ladder sample are identified as alleles by the macro	Use a different injection of the allelic ladder.
	Allelic ladder peaks are too high, causing stutter peaks to be called as allele peaks	Use a shorter injection time, decrease the amount of allelic ladder used, or reanalyze the allelic ladder sample using increased peak amplitude thresholds in the GeneScan® analysis parameters.

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**

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Additional STR references can be found at: www.promega.com/geneticidentity/

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XII. Appendix

XII.A. Composition of Buffers and Solutions

10% ammonium persulfate

Add 0.05g of ammonium persulfate to 500µl of deionized water.

Blue Dextran Loading Solution

88.25%	formamide
15mg/ml	blue dextran
4.1mM	EDTA (pH 8.0)

0.5M EDTA (pH 8.0) stock

186.1g	$Na_2EDTA \bullet 2H_2O$

Add EDTA to 800ml of deionized water with vigorous stirring. Adjust the pH to 8.0 with NaOH (about 20g of NaOH pellets). Dispense into aliquots, and sterilize by autoclaving.

ethidium bromide solution (10mg/ml)

1g	ethidium bromide

Dissolve the ethidium bromide in 100ml of deionized water. Wrap in aluminum foil or transfer the solution to a dark bottle, and store at room temperature.
Caution: Ethidium bromide is a powerful mutagen. Gloves should be worn when working with the dye, and a mask should be worn when weighing it out.

Gold ST★R 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 8.3 at 25°C)
15mM	$MgCl_2$
1%	Triton® X-100
2mM	each dNTP
1.6mg/ml	BSA

TAE 50X buffer (pH 7.2)

242g	Tris base
57.1ml	glacial acetic acid
100ml	0.5M EDTA stock

Add the Tris base and EDTA stock to 500ml of deionized water. Add the glacial acetic acid. Bring to 1 liter with deionized water.

TBE 10X buffer

107.8g	Tris base
7.44g	$Na_2EDTA \bullet 2H_2O$
~55g	boric acid

Dissolve the Tris base and EDTA in 800ml of deionized water. Slowly add the boric acid, and monitor the pH until the desired pH of 8.3 is obtained. Bring the volume to 1 liter with deionized water.

TE^{-4} buffer [10mM Tris-HCl, 0.1mM EDTA (pH 7.5)]

2.21g	Tris base
0.037g	$Na_2EDTA \bullet 2H_2O$

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 7.5 with HCl. Increase volume to 1 liter with deionized water.

XII.B. Related Products

GenePrint® Fluorescent STR Multiplex Systems

Product	Size	Cat.#
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® ES System	100 reactions	DC6731
	400 reactions	DC6730
GammaSTR® Multiplex (Fluorescein)	100 reactions	DC6071
	400 reactions	DC6070
GenePrint® Fluorescent STR Multiplex—CSF1PO, TPOX, TH01, vWA (Fluorescein) (CTTv Multiplex)	100 reactions	DC6301
	400 reactions	DC6300
GenePrint® Fluorescent STR Multiplex—F13A01, FESFPS, F13B, LPL (Fluorescein) (FFFL Multiplex)	100 reactions	DC6311
	400 reactions	DC6310

Not for Medical Diagnostic Use.

GenePrint® Sex Identification Systems

Product	Size	Cat.#
GenePrint® Fluorescent Sex Identification System—Amelogenin (Fluorescein)	100 reactions	DC5171
GenePrint® Fluorescent Sex Identification System—Amelogenin (TMR)	100 reactions	DC6171

Not for Medical Diagnostic Use.

Allelic Ladders

Product	Size	Cat.#
Internal Lane Standard 600	150µl	DG2611
GammaSTR® Allelic Ladder Mix (Fluorescein)	150µl	DG3291
CTTv Allelic Ladder Mix (Fluorescein)	150µl	DG2121
FFFL Allelic Ladder Mix (Fluorescein)	150µl	DG2131

For Laboratory Use.

Accessory Components

Product	Size	Cat.#
PowerPlex® Matrix Standards, 310/377*	50µl (each dye)	DG3640
PowerPlex® Matrix Standards, 3100—Custom*	10µl (each dye)	X3121
PowerTyper™ Macros*	1 CD-ROM	DG3470
Bromophenol Blue Loading Solution**	3ml (3 × 1ml)	DV4371
Fluorescent Ladder CXR, 60–400 Bases**	65µl	DG6221
Gel Tracking Dye**	1ml (4 × 250µl)	DV4361
Gold ST★R 10X Buffer**	1.2ml	DM2411
Mineral Oil	12ml	DY1151
Nuclease-Free Water**	50ml (2 × 25ml)	P1193

*Not for Medical Diagnostic Use.
**For Laboratory Use.

XII.B. Related Products (continued)

Sample Preparation Systems

Product	Size	Cat.#
DNA IQ™ System*	100 reactions	DC6701
	400 reactions	DC6700
AluQuant® Human DNA Quantitation System**	400 determinations	DC1011
	80 determinations	DC1010

*For Laboratory Use.
**Not for Medical Diagnostic Use.

Polyacrylamide Gel Electrophoresis Reagents

Product	Size	Cat.#
Ammonium Persulfate	25g	V3131
TBE Buffer, 10X	1L	V4251
Urea	1kg	V3171

ART® Aerosol-Resistant Tips

Product	Volume	Size (tips/pack)	Cat.#
ART® 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART® 20E Ultramicro Pipet Tip	0.5–20µl	960	DY1061
ART® 20P Pipet Tip	20µl	960	DY1071
ART® GEL Gel Loading Pipet Tip	100µl	960	DY1081
ART® 100 Pipet Tip	100µl	960	DY1101
ART® 100E Pipet Tip	100µl	960	DY1111
ART® 200 Pipet Tip	200µl	960	DY1121
ART® 1000E Pipet Tip	1,000µl	800	DY1131

[a]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

[b]U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048, Korean Pat. No. 290332, Singapore Pat. No. 57050 and Japanese Pat. No. 3602142 have been issued to Promega Corporation for multiplex amplification of STR loci. Other patents are pending.

[c]The purchase of this product does not convey a license to use AmpliTaq Gold® DNA polymerase. You should purchase AmpliTaq Gold® DNA polymerase licensed for the forensic and human identity field directly from your authorized enzyme supplier.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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Printed in USA. Revised 6/07
Part# TMD009

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AF9TMD009 0607TMD009

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